ROLE OF α_1-ADRENERGIC RECEPTORS AND THE EFFECT OF BUNAZOSIN ON THE HISTOPATHOLOGY OF CARDIOMYOPATHIC SYRIAN HAMSTERS OF STRAIN BIO 14.6

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In order to clarify the pathological involvement of the sympathetic nervous system in the development of cardiomyopathy, a receptor-binding study was carried out on cardiomyopathic Syrian hamsters of strain BIO14.6 (BIO) at 21 days (prenecrotic stage); 35–42 days (onset of cardiomyopathy); and 70–84 days of life (early cardiac hypertrophy). The newly developed α_1-blocker (bunazosin hydrochloride) was initially administered at doses of 100 μg/kg or 10 mg/kg to BIO hamsters at 21 days of life and continued for 70 days. At the onset of cardiomyopathy and early cardiac hypertrophy, there was an increase in the number of α_1-receptors in the BIO hamsters compared to controls, but there were no significant changes at the prenecrotic stage. On histopathological examination, 10 mg/kg bunazosin had a significant beneficial effect on cardiomyopathy [area of necrosis 1.38% in untreated vs 0.33% in treated animals; area of calcification 2.70% (untreated) vs 0.60% (treated); area of all myocardial injuries 6.97% (untreated) vs 3.19% (treated)]. However, 100 μg/kg bunazosin had no effect. It was concluded that the increase in the number of α_1-receptors may not be involved in the pathogenesis of cardiomyopathy but that α_1-receptors could be implicated in the later progression of the condition.

THE cardiomyopathic syrian hamster of inbred strain BIO14.6 (BIO) is an animal model of human idiopathic cardiomyopathy. Owing to heredity, this animal is known to develop myocardial necrosis, fibrosis, and calcification at 30–40 days of life, followed by cardiac hypertrophy and congestive heart failure.[1,2] The Ca^{2+} overload state in the myocardial cell, which is evident even in the prenecrotic stage in this animal, is believed to play an important role in the development of cardiomyopathy,[3–5] but the mechanism of this overload has yet to be elucidated.

Key words:
α_1-adrenergic receptor
Cardiomyopathic syrian hamster
Bunazosin

Karliner et al[6] reported that the response to α-receptor stimulation was enhanced in the hearts of 6 and 12–14 months old cardiomyopathic hamsters and that the numbers of cardiac α_1- and β-receptors are higher in cardiomyopathic hamsters than in controls.

It has also been reported that the α_1-blocker prazosin hydrochloride is beneficial in countering cardiomyopathy in hamsters.[7] These reports[6,7] suggest that the sympathetic nervous system may be involved in the development of cardiomyopathy in BIO hamsters; however, it is not yet known whether such involvement occurs in the early stage of cardiomyopathy.

In light of these reports, it was decided to

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TABLE I [^{125}I]HEAT (α_1-RECEPTOR)

	21 days old		35–42 days old		70–84 days old	
	control (n = 12)	BIO 14.6 (n = 12)	control (n = 7)	BIO 14.6 (n = 7)	control (n = 6)	BIO 14.6 (n = 6)
Bmax (fmol/mg protein)	30.4 ± 2.2	30.0 ± 4.5	21.3 ± 4.3	25.3 ± 2.5*	14.5 ± 2.6	18.7 ± 3.6*
Kd (pM)	204.1 ± 70.6	198.6 ± 99.0	141.3 ± 52.3	146.0 ± 8.5	152.1 ± 24.6	161.6 ± 32.3

Values are mean ± s.d.
Bmax and Kd values obtained in the α_1-receptor binding study in Syrian hamster BIO14.6 and controls at 21, 35–42 and 70–84 days of life.
**Indicates a statistically significant difference between BIO14.6 and age-matched control groups ($p < 0.05$).*

TABLE II [^{125}I]CYP (β-RECEPTOR)

	21 days old		35–42 days old		70–84 days old	
	control (n = 12)	BIO 14.6 (n = 12)	control (n = 7)	BIO 14.6 (n = 7)	control (n = 6)	BIO 14.6 (n = 6)
Bmax (fmol/mg protein)	31.4 ± 8.0	31.6 ± 8.9	30.6 ± 1.3	36.9 ± 4.5*	23.7 ± 3.6	32.0 ± 2.7*
Kd (pM)	16.8 ± 3.3	13.3 ± 8.9	16.6 ± 3.6	19.0 ± 9.6	22.7 ± 7.9	20.3 ± 3.6

Values are mean ± s.d.
Bmax and Kd values obtained in the β-receptor binding study in Syrian hamster BIO14.6 and controls at 21, 35–42 and 70–84 days of life.
**Indicates a statistically significant difference between BIO14.6 and age-matched control groups ($p < 0.05$).*

investigate whether α_1-receptors are involved in the early stage of cardiomyopathy, using BIO hamsters at 21 days (prenecrotic stage); 35–42 days (onset of cardiomyopathy); and 70–84 days of life (early cardiac hypertrophy). The numbers of cardiac α_1- and β-receptors were determined by a radioligand-binding assay method. The newly developed α_1-blocker bunazosin hydrochloride was administered to hamsters at the prenecrotic stage for 70 days in order to determine its effects on the histopathology of cardiomyopathy.

MATERIALS AND METHODS

Animals

BIO hamsters of either sex used in the study were purchased from the Central Institute for Experimental Animals (1430, Nogawa, Kawasaki, Kanagawa, 213 Japan). Age-matched golden hamsters were obtained from the Inoue Experimental Animals Center as controls in the receptor-binding study.

Receptor-binding Study

Cardiac Membrane Preparation

The difference in receptor binding was determined in the BIO and golden hamsters at 21 days; 35–42 days; and 70–84 days of life.

The animals were sacrificed by cervical dislocation, and their hearts removed. For the group of 21-day-old animals, three hearts were used as one specimen, and one heart was used as one specimen for the other two groups of 35–42-day-old and 70–84-day-old animals. The heart was trimmed of the atrium and large vessels, and the right and left ventricles were cut into small pieces using scissors. The tissue specimen was homogenized in 50 ml/g wet tissue of cold homogenate buffer (0.25 mol/l sucrose, 5 mmol/l tris/HCl, pH 7.4), using a polytron and Potter-Elvehjem homogenizer. The homogenate was

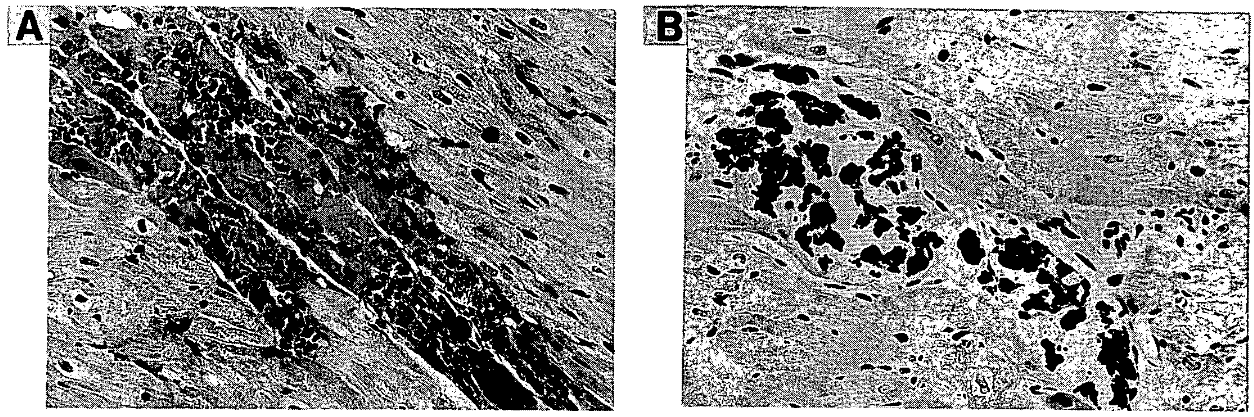

Fig.1. Enlarged photographs of lesions of (A) necrosis, (B) calcification, (C) fibrosis in BIO14.6 cardiomyopathic hamsters.
(A) Muscle cells in the center show loss of striations with increased eosinophilia of cytoplasm. There are inflamatory cells infiltration and bleeding. (Hematoxylin and Eosin)
(B) Calcium deposits are evident as dark dots on the damaged muscles. (von Kossa's stain)
(C) There are dense collagen fibers (arrowheads) running between muscle fibers. (Mallory-Heidenhain)

centrifuged at 500*g* at 4°C for 10 min, and the supernatant at 30 000*g* at 4°C for 10 min. The resulting pellets were suspended in cold incubation buffer (50 mmol/l tris/HCL, pH 7.4) and washed by centrifuging at 30 000*g* at 4°C for 10 min. The last pellet was suspended in 20 ml/g wet tissue of 50 mmol/l tris/HCL buffer, pH 7.4. The specimens were assayed for protein according to the method of Lowry et al.[8]

Assay for Cardiac α_1-Receptors

Aliquots (50 μl) of cardiac membrane homogenates were incubated with six different concentrations of [^{125}I]HEAT [dl-2-[β-(3-[^{125}I] iodo-4-hydroxyphenyl)-ethyl-aminoethyl] tetralone] ranging from 0.012 to 0.400 nmol/l in a total volume of 500 μl containing incubation buffer with or without 10 μmol/l prazosin. The incubation was performed at 30°C for 90 min and terminated by the addition of 3 ml of 20 mmol/l phosphate buffer, pH 7.0. Membrane-bound [^{125}I]HEAT was trapped at the end of the incubation period by rapid vacuum filtration of the incubation mixture through a Whatman glass fibrefilter (GF/C).

The filter was immediately rinsed with three aliquots (3 ml) of 20 mmol/l phosphate buffer, pH 7.0, and the radioactivity trapped on the filter was measured in a Hitachi automatic well counter.

Non-specific binding, defined as the binding of [^{125}I]HEAT in the presence of 10 μmol/l prazosin, was subtracted from the total binding to obtain the specific. The binding assay was carried out in duplicate.

Assay for Cardiac β-Receptors

The binding assay for β-receptors was performed in a similar way to that for α_1-receptors, except that the incubation, with six different concentrations of [^{125}I]CYP [(−)-3-[^{125}I] iodocyano pindolol] ranging from 0.003 to 0.250 nmol/l, with or without 10 μmol/l propranolol, was carried out at 30°C for 90 min. Non-specific binding was defined as the binding of [^{125}I]CYP in the presence of 10 μmol/l propranolol.

Histological Study

BIO hamsters at 21 days of life were used in the histological study.

Bunazosin hydrochloride dissolved in distilled water at a concentration of 0.05 mg/ml was administered intraperitoneally at a dose of 100 μg/kg every 12h to experimental group I. A 5 mg/ml bunazosin hydrochloride solution in

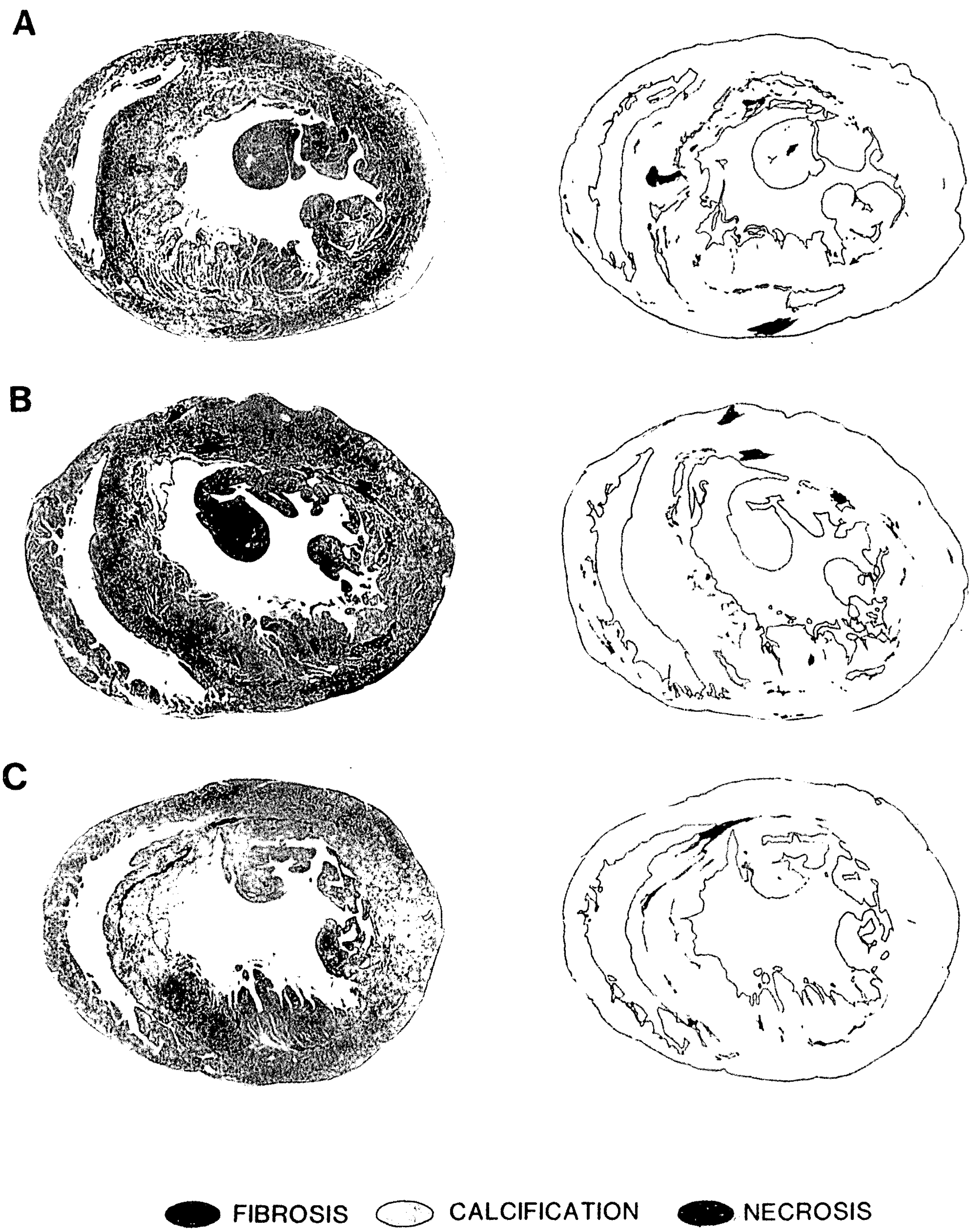

Fig.2. Sites of myocardial injury in BIO14.6 cardiomyopathic hamsters; (A) non-treated group, (B) group I receiving a low dose of bunazosin and (C) group II receiving a high dose of bunazosin. On the left are shown photographs of stained tissue sections (with Hematoxylin and Eosin) and on the right are shown the sites of injury which were traced from the photographs to calculate the all- and individual-injury areas.
Blue color portions display sites of fibrosis, orange color portions display sites of cacification, green color portions display sites of necrosis.

distilled water was administered to experimental group II at a dose of 10 mg/kg every 12h. The non-treated group received an appropriate volume of distilled water. Administration was started at 21 days of life and continued for 70 days. The animals were sacrificed by cervical

TABLE III HISTOLOGICAL RESULTS OF THE TREATMENT WITH BUNAZOSIN

	Groups		
	Non-treated (n = 5)	Bunazosin I (100 μg/kg) (n = 7)	Bunazosin II (10 mg/kg) (n = 5)
Area of all myocardial injuries			
mm^2	8788 ± 1957	6608 ± 981	4204 ± 724*
%	6.97 ± 1.34	5.33 ± 0.80	3.19 ± 0.59*
Area of necrosis:			
mm^2	1737 ± 457	1873 ± 600	444 ± 121*
%	1.38 ± 0.35	1.42 ± 0.46	0.33 ± 0.09*
Area of calcification:			
mm^2	3400 ± 920	2064 ± 235	850 ± 247*
%	2.70 ± 0.64	1.66 ± 0.18	0.60 ± 0.17**
Area of fibrosis:			
mm^2	3650 ± 934	2671 ± 349	2909 ± 499
%	2.89 ± 0.67	2.24 ± 0.39	2.21 ± 0.41

Values are mean ± s.e.m.
Area and area ratios of myocardial injuries to whole heart muscle section.
**Indicates a statistically significant difference between treated and non-treated groups ($p < 0.05$) and*
***indicates a statistically significant difference between treated and non-treated groups ($p < 0.01$).*

dislocation 32h after the last dosing.

The histological study was conducted as previously reported.[9] Briefly, the hearts were sliced at the center into pieces 5 mm thick, which were immediately fixed in 10% buffered formalin. After 24–48h of fixation, the sections were embedded in solid paraffin. The pieces of tissue were sliced into sections 3 μm thick and stained with haematoxylin and eosin, Mallory-Heidenhain triple stain, and von Kossa's stain. The tissue specimens were photographed at ×70 magnification after each stain.

The total area of heart muscle and the area of necrosis, fibrosis and calcification in the heart muscle tissue specimens were traced from the photographs and measured, using a image analyser system, video plan (Konton, West Germany).

The ratios of the areas of necrosis, fibrosis and calcification to the area of the whole heart muscle section were compared for groups I, II and the non-treated group.

Statistical Analysis

Data obtained in the receptor binding study are reported as mean ± s.d., and data obtained in the histological study, as mean ± s.e.m.

Taking p = 0.05 as the limit of significance, the data were assessed by the Student t-test for the significance of differrences.

RESULTS

Receptor Binding Study (Tables I, II)

The total numbers of α_1-receptors in BIO hamsters were significantly increased in animals at 35–42 and 70–84 days of life compared to control, but showed no difference between the animals at 21 days of life and controls. Furthermore, there was no significant difference in α_1-receptor affinity between BIO hamster and control groups at any one of the three ages.

The total numbers of β-receptors in BIO hamsters were significantly increased in animals at 35–42 and 70–84 days of life compared to control, but showed no significant difference between the animals at 21 days of life and controls. Furthermore, there was no significant difference in β-receptor affinity between BIO hamsters and control groups at any one of the three ages.

Histological Study

Figure 1 shows enlarged photographs of necrosis, fibrosis and calcification. Fig. 2 shows the area of necrosis, fibrosis and calcification in typical examples from groups I, II and the non-treated group.

In group II, which received bunazosin at a high dose, the areas of all myocardial injuries, necrosis and calcification, and the corresponding area ratios were significantly reduced compared to the non-treated group; this was not true of group I, which received bunazosin at a low dose (Table III).

In groups I and II, the areas of fibrosis and fibrosis area ratios were not significantly reduced compared to the non-treated group (Table III).

DISCUSSION

It has been reported on the basis of evidence from light micrographs, that there are lesions in the hearts of BIO hamsters at 30–40 days of life or later[1,2] and that intra-cellular Ca^{2+}-overload exists prior to the development of lesions.[3] Since Ca^{2+}-antagonists improve cardiomyopathy, a disturbance in calcium metabolism is assumed to play an important role in its development.[9–11] However, the mechanism by which this disturbance occurs has yet to be elucidated.

Wagner et al.,[12] who found that the number of Ca^{2+}-antagonist receptors was increased in cardiomyopathic hamsters even at 30 days of life, proposed that an increase in the number of voltage-dependent calcium channels might be involved in the pathogenesis of cardiomyopathy. However, it is known that large doses of adrenergic agonists, including norepinephrine, give rise to myocardial changes similar to cardiomyopathy.[13,14] Karliner et al.[6] reported that, in the hearts of cardiomyopathic hamsters at 6 and 12–14 months of life, the response to α-receptor stimulation was enhanced and the numbers of α_1- and β-receptors were increased. This report[6] suggests that the sympathetic nervous system is at fault in cardiomyopathic hamsters.

There are also reports[10,15] that cardiomyopathy in hamsters does not improve on the administration of the β-blocker, propranolol; in contrast, the condition is prevented from progressing by the administration of the α_1-blocker, prazosin.[7] These findings suggest that α_1-receptors are more deeply implicated in the development of cardiomyopathy in hamsters than β-receptors.

In our receptor-binding study, the numbers of α_1- and β-receptors were increased in BIO hamsters at 35–42 days (onset of cardiomyopathy) and at 70–84 days of life (early cardiac hypertrophy). These results are in agreement with those of other investigators;[6] however, in animals at 21 days of life (prenecrotic stage) this was not the case. Unlike Ca^{2+}-antagonist receptors,[12] the number of α_1-receptors did not increase in BIO hamsters at 21 days of life. This fact seems to rule out the possibility that an increase in the number of α_1-receptors might be involved in the pathogenesis of cardiomyopathy.

α_1-Receptors promote the flow of Ca^{2+} into the myocardial cell[16] and enhance Ca^{2+}-related myofibre sensitivity.[17] There is a report[18] that in smooth muscle, Ca^{2+} is released from the Ca^{2+} store in the cell via phosphatidyl inositol response. It is therefore conceivable that α_1-receptor stimulation enhances the state of Ca^{2+}-overload, thereby causing cardiomyopathy to progress. In this histopathological study, it was found that the α_1-blocker bunazosin hydrochloride at a dose of 10 mg/kg inhibited the progression of cardiomyopathy. This finding suggests that α_1-receptor stimulation is implicated in the later progression of the condition.

There are reports[19–21] that the increase in the number of adrenergic receptors may represent an adaptive change in response to catecholamine depletion in pressure overload left ventricular hypertrophy produced by constriction of the aorta. On the other hand, Sole et al.[22] reported that myocardial tissue levels of norepinephrine were elevated in cardiomyopathic hamsters at 18–20 days and 45–65 days of life. The increase in α_1-receptor numbers found in BIO hamsters at 35–42 and 70–84 days of life dose not represent an adaptive change in response to catecholamine depletion, because myocardial tissue levels of norepinephrine are elevated. When the increase in the number of α_1-receptors at the onset of cardiomyopathy and in early cardiac hypertrophy is considered together with the reported elevation in myocardial tissue levels of norepinephrine, it seems tenable to hold that α_1-receptor stimulation is concerned with the later progression of cardiomyopathy in connection with sympathetic activity enhancement.

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